DATA EVALUATION RECORD

PICOXYSTROBIN (DPX-YT669)

Study Type: OPPTS 870.3800 [§83-4]; Multigeneration Reproduction Study in Rats

Work Assignment No. 7-01-256 E (MRID 48073739)

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DATA EVALUATION RECORD

STUDY TYPE: Reproduction and Fertility Effects Study - rat; OPPTS 870.3800 [§83-4]; OECD 416.

PC CODE: 129200 **DP BARCODE:** D378236
TXR#: 0056696 **SUBMISSION #:** S873059

TEST MATERIAL (PURITY): Picoxystrobin (99.3% a.i.)

SYNONYMS: DPX-YT669; Methyl (α*E*)-α-(methoxymethylene)-2-[[[6-(trifluoromethyl)-2-pyridinyl]oxy] methyl]benzeneacetate

CITATION: Barnett, Jr., J.F. (2010) Oral (diet) two-generation (one litter per generation) reproduction toxicity study of Picoxystrobin (DPX-YT669) technical in rats. Charles River Laboratories, Preclinical Services, Horsham, PA. Laboratory Project ID: AUV00036, April 14, 2010. MRID 48073739. Unpublished.

SPONSOR: DuPont Haskell Global Centers for Health and Environmental Sciences, 1090 Elkton Road, Newark, DE

EXECUTIVE SUMMARY: In a two-generation reproduction toxicity study (MRID 48073739), Picoxystrobin (99.3%; Lot # DPX-YT669-028) was administered continuously in the diet to 30 Sprague-Dawley (Crl:CD[SD]) rats/sex/dose group for two consecutive generations at dietary levels of 0, 75, 300, 1000, or 2500 ppm (equivalent to 0/0, 4.2/5.4, 16.9/21.7, 55.5/70.3, and 137.5/173.4 mg/kg/day in males/females, respectively). The P generation animals (30/sex/dose group) were exposed to the test diets beginning at approximately 8-9 weeks of age, for at least 10 weeks prior to mating to produce the F1 litters. F1 offspring selected to be parents of the next generation (30/sex/dose group) were fed the same test diet concentrations as their parents. F1 parents were fed the test diets for at least 10 weeks prior to mating to produce the F2 generation. The F2 offspring were terminated after weaning.

Parent animal toxicity: The treatment-related effects produced by picoxystrobin were mainly seen in the 2500 ppm P and F1 parental rats. The effects were on body weights, body weight gains, food consumption, food efficiency changes, organ weight changes, and histopathology. The effects on body weights, body weight gains, food consumption, and food efficiency are tabulated below, and the details are shown in Tables 3a, 3b, 4 and 5.

Percent Change Relative to the Controls (%) in P & F1 Generation Rats at 2500 ppm								
Periods	**Body Weights**		**Body Weight Gains**		**Food Consumption**		**Food Efficiency**	
	Males	Females	Males	Females	Males	Females	Males	Females
	P Generation							
Pre-mating	↓4-7%	↓5-7%	↓18%	↓26%	↓7%	↓8%	↓11%	↓19
Gestation		↓7-8%		↓8%		↓10%		–
Lactation		↓7-8%		↑223%		↓16-20%		–
	F1 Parental Rats							
Pre-mating	10-26%↓	9-27%↓	↓8%	↓5%	↓9%	↓9%	–	↑5%
Gestation		↓8%		↓9%		↓8%		–
Lactation		↓6-10%		↑304%		↓12% at LD 11-15		↑64%

Additionally at 2500 ppm, the following differences in organ weights compared to controls were noted in the P generation females: (i) absolute and relative (to body and to brain weight) liver weights were increased by 6-10%; (ii) absolute and relative (to body and to brain weight) thymus weights were decreased by 29-32%; (iii) absolute and relative (to body and to brain weight) non-gravid uterus weights were decreased by 30-32%; (iv) absolute and relative (to body and to brain weight) pituitary weights were decreased by 13-20%; (v) absolute brain weight was decreased by 3%; and (vi) absolute and relative (to body weight) right ovary weights were decreased by 13% each. Findings in the P generation males were limited to increased relative (to body weight) liver weight (incr. 10%) at this dose. Microscopic findings were limited to minimal to moderate lymphoid atrophy in the thymus in the 2500 ppm females (11/30 treated vs. 0/30 controls).

The LOAEL for parental toxicity is 2500 ppm (137.5/173.4 mg/kg/day in males/females, respectively) based on decreases in body weight, body weight gain, and food consumption in the P and F1 generation during pre-mating; increased body weight gains in the P and F1 females at the end of the lactation period; organ weight differences; and minimal to moderate lymphoid atrophy in the thymus in P generation females. The NOAEL is 1000 ppm (55.6/70.3 mg/kg/day in males/females, respectively).

*Offspring toxicity***:** There were no treatment-related effects on: mortality; clinical signs; live birth, viability, and lactation indices; or pup sex ratio for either generation. There were no treatment-related gross or microscopic findings in the F1 or F2 pups.

At 2500 ppm, overall (PND 1-22) pup body weight gains (calculated by reviewers) were decreased in both the F1 and F2 generations by 17-26%. Mean pup body weights/litter at this dose were decreased by 13-23% on PND 8, 15, and 22 in the F1 generation and by 12-14% on PND 15 and 22 in the F2 generation. Organs weights such as thymus, spleen, and thyroid were decreased in F1 and F2 pups.

The LOAEL for offspring toxicity is 2500 ppm (137.5/173.4 mg/kg/day in males/females, respectively) based on decreased mean pup body weights/litter and body weight gains in the F1 and F2 generations and decreased organ weights including spleen, thymus, and thyroid. The NOAEL is 1000 ppm (55.6/70.3 mg/kg/day in males/females, respectively).

Reproductive toxicity: There were no effects of treatment in either generation on: estrous cycle; sperm parameters; mating, fertility, litter sizes, sex ratio, pup birth weights, or gestation indices; pre-coital interval; or gestation duration.

The LOAEL for reproductive toxicity was not observed. The NOAEL is 2500 ppm (137.5/173.4 mg/kg/day in males/females, respectively) (HDT).

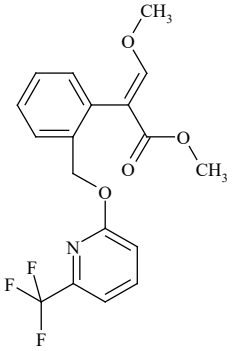
This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.3800; OECD 416) for a two-generation reproduction study in the rat.

COMPLIANCE: Signed and dated GLP Compliance and Quality Assurance statements were provided. Data Confidentiality and Flagging statements were provided; however, they were not signed.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Picoxystrobin

Description:	Not reported
Lot #:	DPX-YT669-028
Purity:	99.3% a.i.
Compound stability:	The test material was shown to be stable in the diet for up to 30 days at room temperature or frozen.
CAS # of TGAI:	117428-22-5
Structure:	

2. Vehicle: Diet

3. Test animals

Species:	Rat
Strain:	Crl:CD(SD)
Age at study initiation:	(P) Approximately 8-9 weeks
Mean body weight at initiation of treatment:	(P) Males: 364.8-367.8g; Females: 225.0-229.1 g
Source:	Charles River Laboratories, Inc. (Raleigh, NC)
Housing:	During pre-mating, all rats were individually housed in stainless steel, wire-bottomed cages. During cohabitation, one male and one female were housed in the male rat's cage. Beginning no later than gestation day (GD) 20, females were individually housed in nesting boxes until they either delivered naturally or were sacrificed on GD 25. Dams with their litters were individually housed in a common nesting box until weaning.
Diet:	Certified Rodent Diet® #5002 (PMI Nutrition International, St. Louis, Mo), *ad libitum*
Water:	Reverse osmosis treated tap water, *ad libitum*. Chlorine was added as a bacteriostat.
Environmental conditions:	**Temperature** 19-25ºC **Humidity** 30-70% **Air changes** At least 10/hour **Light cycle** 12 hrs light : 12 hrs dark
Acclimation period:	Not reported

B. PROCEDURES AND STUDY DESIGN

1. In-life dates: P1 generation Start: April 30, 2008 End: September 6, 2008
F1 generation Start: August 22, 2008 End: January 2, 2009

2. **Mating procedure:** Mating was accomplished by co-housing one female with one male for up to 21 consecutive days. Sibling matings were excluded in the F1 parents. Evidence of mating was assessed by checking for copulation plugs and by examining a vaginal smear prepared from each female for the presence of spermatozoa. The day on which positive evidence of mating was found was designated as GD 0. Once mating had occurred, the males and females were separated. Females not mated within the first 14 days of cohabitation were assigned alternate proven males from the same dose group, and remained paired for a maximum of seven additional days. Females not mated after the completion of the 21-day mating period were considered to be at GD 0 and were assigned to individual housing.

3. **Study schedule:** P generation rats (30/sex/dose group) were fed the test diets for at least 70 days prior to mating to produce the F1 litters. Following the mating and gestation periods, dams were allowed to deliver and rear the F1 litters until weaning on LD 21. Following weaning, F1 offspring (30/sex/dose group) were selected to be parents of the next generation and were fed the same test diet concentration as their dam for at least 70 days prior to mating to produce the F2 generation. The F2 pups were terminated on post-natal day (PND) 22.

4. **Animal assignment:** Before commencement of treatment, the P animals were assigned to the dose groups shown in Table 1 using a computer-generated (weight-ordered) randomization procedure. Following weaning on PND 22, F1 offspring (30/sex/dose group) were selected to be parents of the next generation by selecting one pup/sex/litter, where possible, using a table of random numbers.

TABLE 1. Animal assignment [a]

Test group	Dose (ppm) [b]	Animals/group			
		P Males	P Females	F_1 Males	F_1 Females
Control	0	30	30	30	30
Low	75	30	30	30	30
Mid-low	300	30	30	30	30
Mid-high	1000	30	30	30	30
High	2500	30	30	30	30

a Data were obtained from pages 43 and 44 of the study report.
b Exposure to the test substance was continuous throughout the study.

5. **Dose-selection rationale:** The dietary concentrations listed in Table 1 above were provided by the Sponsor based on the results of previous studies. No further details were provided.

6. **Test diet preparation and analysis:** Dietary formulations were prepared as needed, stored at room temperature, and were used within 30 days from the date of preparation. Details of the formulation procedure were not provided. Stability for up to 30 days at room temperature or frozen was verified in the 75 and 2500 ppm dietary formulations prepared for the first week of dosing. Homogeneity (top, middle, bottom) analyses were conducted at all concentrations prepared for the first week of dosing and again at Week 6 (due to the increase in preparation size using a new blender). Concentration analyses were conducted on samples taken from all dietary concentrations prepared at the beginning, middle, and end of the dosing period.

Results

Homogeneity (%RSD): 1-10%, excluding the 75 ppm sample from Week 6 (16%RSD)

Stability (% nominal concentration): 98.3-110% after 30 days at room temperature
103.6-109.2% after 30 days frozen

Concentration:

Dose (ppm)	% Nominal
75	86.9-104.9
300	93.3-108.3
1000	92.0-112.0
2500	99.2-113.6

Homogeneity analyses of the 75 ppm formulation from June 6, 2008 (Week 6) indicated a RSD of 16%, which was outside the acceptable range (0-10%). This deviation was a result of the bottom stratum (123.2% of nominal), while the top and middle strata were 93.7-96.1% of nominal. The analytical data indicated that the mixing procedure was marginally adequate and that the variation between nominal and actual dosage to the study animals was acceptable.

7. **Dosage administration:** The test material was administered in the diet continuously throughout the study (i.e., P generation adults were fed the test diets *ad libitum* beginning 70 days prior to mating, and the selected F1 adults were fed the same test diet concentrations as their parents beginning on PND 21.

C. OBSERVATIONS

1. **Parental animals:** All animals were observed at least twice daily throughout the study for mortality and at least weekly during the acclimation and dosing periods for clinical signs of toxicity. In the males, body weights were recorded at least weekly during the acclimation and dosing periods and at termination, and food consumption was recorded weekly during the dosing period. In the females, body weights were recorded at least weekly during the acclimation and dosing periods, on GD 0, 7, 14, 21, and 25, and on LD 1, 5, 8, 15, and 22 (terminal body weight). Additionally in the females, food consumption was recorded at least weekly during the acclimation and dosing periods, on GD 0, 7, 14, 18, 21, and 25, and on LD 1, 5, 8, 11, and 15. For both sexes, food efficiency was calculated as the (average g/day of body weight gain divided by the average g/day of food consumption) multiplied by 100 and presented for the same day ranges as food consumption.

   Estrous cycling was evaluated by examination of vaginal cytology for 21 days prior to scheduled cohabitation and then until positive evidence of mating was observed. The estrous cycle was also evaluated by examination of vaginal cytology prior to sacrifice on LD 22. Sperm parameters were measured in all P and F1 males. A homogenate was prepared from the left cauda epididymis to determine sperm concentration (sperm per gram of tissue). Motility was evaluated by collection of a sample from the left vas deferens. Testicular and epididymal sperm counts were included in sperm evaluations. The remaining portion of the

left cauda epididymis was used to manually evaluate sperm morphology. Sperm morphology evaluations included determination of the percentage of normal sperm in a sample of at least 200, and qualitative evaluation of abnormal sperm, including such categories as abnormal head, abnormal tail, and abnormal head and tail.

2. Litter observations: The following litter parameters (X) were recorded (Table 2):

TABLE 2. F_1 / F_2 Litter Observations [a]

Observation	Time of observation (post-natal day)					
	Day 1	Day 5 (pre-cull)	Day 5 (post-cull)	Day 8	Day 15	Day 22
Number of live pups	X	X	X	X	X	X
Number of dead pups	X	X	X	X	X	X
Pup weight	X	X	X	X	X	X
Sex of each pup (M/F)	X	X	X	X	X	X
External alterations	X	X	X	X	X	X

a Data were obtained from pages 45-47 and from Tables B22 and D23 on pages 226-228 and 564-566 of the study report.

Litters were observed daily for mortality and clinical signs of toxicity. Wherever possible, any offspring found dead were examined externally and internally. On PND 5, litters were standardized to 8 pups each (4/sex where possible). Sexual maturation was recorded for the F1 offspring during the post-weaning period by examining females for vaginal patency beginning on PND 28 and males for preputial separation beginning on PND 39; age and body weight at criterion were recorded.

3. Postmortem observations

a. Parental animals: All surviving P and F1 animals were euthanized by inhalation of carbon dioxide and subjected to a gross necropsy. The males were terminated after completion of the cohabitation period. The dams were euthanized on LD 22, after their respective litters were weaned. Females that failed to deliver a litter were euthanized on GD 25 and the uteri were stained with 10% ammonium sulfide to confirm the absence of implantation sites.

The following tissues from all P and F1 parents were collected (X) and weighed (XX). Organ-to-body weight and organ-to-brain weight ratios were calculated.

	FEMALE REPRODUCTIVE		MALE REPRODUCTIVE		BOTH SEXES
XX	Ovaries	XX	Testes	XX	Adrenals
XX	Uterus with oviducts and cervix	XX	Epididymides	XX	Brain
X	Vagina	XX	Prostate	XX	Kidneys
X	Mammary gland	XX	Seminal vesicles with coagulation gland	XX	Liver
				XX	Pituitary gland
				XX	Spleen
				XX	Thymus
				XX	Thyroid with parathyroids [a]
				X	Gross lesions

a Weighed after fixation

The tissues listed above were immediately fixed in neutral buffered 10% formalin, with the exception of the testes which were initially fixed in Bouin's solution for 48 to 96 hours and subsequently retained in neutral buffered 10% formalin. The thymus, vagina, uterus, ovary, oviduct, and cervix from all P generation females were routinely processed, stained with hematoxylin and eosin, and examined microscopically. A peer review of the gross and microscopic pathology was also performed.

Findings were assigned a severity grade on a four point scale (minimal, mild, moderate, or marked).

b. Offspring: Pups ≤14 days old were euthanized by an intraperitoneal injection of sodium pentobarbital; pups ≥15 days old were euthanized by carbon dioxide asphyxiation. Pups found dead on PND 1-5 were preserved in Bouin's solution for possible future evaluation; pups found dead on PND 6-22 were preserved in neutral buffered 10% formalin. All pups culled on PND 5 or not selected for continued observation on PND 22 were examined for gross lesions. The necropsy included a single cross-section of the head at the level of the frontal-parietal suture and examined for apparent hydrocephaly. Culled PND 5 pups with gross lesions were preserved in Bouin's solution for possible future evaluation. In the unselected PND 22 pups, the spleen, thymus, thyroid, adrenals, uterus, and brain from one randomly selected pup/sex/litter were weighed and retained in neutral buffered 10% formalin for possible future evaluation. The remaining pups were discarded with further examination.

D. DATA ANALYSIS

1. Statistics: The data were analyzed using the following statistical methods.

Parameter	Statistical Procedures
Continuous data (e.g., maternal body weights, body weight gains, and food consumption) Sperm motility data	Bartlett's test was performed. If the result was not significant ($p>0.001$), then ANOVA was performed. When ANOVA was significant ($p\leq0.05$), Dunnett's test was conducted. If Bartlett's test revealed heterogeneity of variances, the Kruskal-Wallis test was performed when 75% or fewer of the scores in all the groups were tied, and when this test result was significant ($p\leq0.05$), Dunn's test was conducted. When more than 75% of the scores in any group were tied, Fisher's Exact Test was used to compare the proportion of ties in the groups.
Count data	The Kruskal-Wallis test was performed when 75% or fewer of the scores in all the groups were tied, and when this test result was significant ($p\leq0.05$), Dunn's test was conducted. When more than 75% of the scores in any group were tied, Fisher's Exact Test was used to compare the proportion of ties in the groups.
Clinical observations and other proportional data	Contingency tables using the Variance Test for Homogeneity of the Binomial Distribution were used.

Statistical significance was denoted at $p<0.05$ or $p<0.01$. The reviewers consider the analyses used to be appropriate.

2. Indices

Reproductive indices: The following reproductive indices were calculated by the

performing laboratory from breeding and parturition records of animals in the study:

Mating index (%) = # animals mated/ # animals paired x 100

Fertility index (%) = # animals that achieved a pregnancy/ # animals paired x 100

Gestation index (%) = # live litters born/# pregnant females x 100

Offspring viability indices: The following offspring indices were calculated by the performing laboratory from lactation records of litters in the study:

Live birth index (%) = # live pups on PND 1/ total # pups on PND 1 x 100

Viability index (%) = # live pups on PND 5 (pre-cull)/ # live pups on PND 1 x 100

Lactation index (%) = # live pups on PND 22/ # live pups on PND 5 (post-cull) x 100

3. **Historical control data:** Historical control data for maternal necropsy findings, delivery observations, and sexual maturation were provided on pages 1450-1459 of the study report.

II. RESULTS

A. PARENTAL ANIMALS

1. Mortality and clinical signs

a. **Mortality:** No treatment-related mortalities were observed in the P generation males or females at any dose. One 2500 ppm male (#16944) was humanely sacrificed on Day 92 due to adverse clinical signs (chromodacryorrhea in both eyes, misaligned upper incisors, decreased motor activity, ataxia, vocalization, ptosis, pale eyes, ears and extremities, bradypnea, red substance in the cage pan and on the penis and fur). At necropsy, the mucosal surface of the fundic region of the stomach was discolored yellow; the bladder contained approximately 0.5 mL of dark red fluid and gelatinous material; extreme dilation in the pelvis of the right kidney and the kidney contained red gelatinous material and white firm caseous material; and the right kidney was surrounded by edematous material. This death was not considered to be related to treatment because the renal lesions found in this animal are known to occur spontaneously and no other rats in this group had similar findings. Additionally, two P generation females (one each at 300 and 1000 ppm) were found dead on GD 20 and 22, respectively. These deaths were not dose-dependent and were considered to be unrelated to treatment. All other P generation females survived to scheduled sacrifice.

In the F1 generation, one 2500 ppm male (#4540) was found dead on PND 26. Clinical signs observed included mild dehydration and pale extremities. Body weight for this animal was comparable with other rats in this exposure group. At necropsy, the lungs were mottled red and dark red; all other tissues appeared normal. As this was the only F1 male pup that died, this death was considered incidental. Additionally, one 2500 ppm female (#4678) was humanely sacrificed on PND 27 due to adverse clinical signs (ataxia, decreased motor

activity, chromorhinorrhea, both eyes appearing red and sunken in the head, pale extremities, coldness to the touch, severe dehydration, thin appearance, hunched posture, tachypnea, and scant feces). The body weight recorded for this animal on PND 23 was unremarkable. All tissues appeared normal at necropsy. As this was the only F1 female pup that died, this death was considered incidental.

b. Clinical signs of toxicity: Aside from the clinical signs associated with the mortalities mentioned above, there were no remarkable clinical observations in the P generation males or females. In the F1 generation males, the number of rats displaying mild dehydration was increased ($p \leq 0.01$, 6/30 treated vs. 1/30 controls) at 2500 ppm. Aside from this mild dehydration and the clinical signs associated with the mortalities mentioned above, there were no remarkable clinical observations in the F1 generation males or females.

2. Body weight, body weight gain, and food consumption

a. Pre-mating: Pre-mating body weights and body weight gains for the P and F1 generations are presented in Table 3a. In the P generation at 2500 ppm, overall pre-mating (Days 1-70) body weight gains were decreased ($p \leq 0.01$) by 18% in the males and by 26% in the females, compared to controls. Additionally at this dose, body weights were decreased ($p \leq 0.05$) throughout the pre-mating period by 4-7% in the males and 5-7% in the females. In the F1 generation at 2500 ppm, overall pre-mating (Days 23-86) body weight gains were decreased ($p \leq 0.01$) by 8% in the males and by 5% (not statistically significant [NS]) in the females. Additionally at this dose, body weights were decreased ($p \leq 0.01$) throughout the pre-mating period by 10-26% in the males and by 9-27% in the females.

TABLE 3a. Mean (±SD) body weights and body weight gains (g) during pre-mating. [a]						
Observation	Days	Dose (ppm)				
		0	75	300	1000	2500
P generation males						
Body weight	1	364.8±18.0	367.8±15.8	367.8±16.1	366.5±16.3	367.0±16.2
	8	402.4±25.2	405.6±17.6	401.9±21.5	399.5±18.0	387.3±18.4** (↓4)
	64	567.8±58.0	564.9±45.8	558.3±45.9	543.0±43.6	527.6±43.0** (↓7)
	70	572.3±56.2	573.7±47.9	568.5±48.1	551.5±45.0	537.1±45.3* (↓6)
Body wt gain	1-70	207.5±45.0	205.9±41.0	200.7±42.6	185.0±41.4	170.2±34.7**(↓18)
P generation females						
Body weight	1	227.5±9.3	229.1±10.2	225.7±8.5	225.0±7.6	227.2±8.0
	8	244.0±13.6	247.9±11.9	245.1±14.5	238.3±9.6	232.8±9.7** (↓5)
	70	303.8±24.5	314.0±26.1	313.0±30.4	296.9±22.4	283.9±16.5** (↓7)
Body wt gain	1-70	76.3±21.5	84.9±21.2	87.3±25.4	71.9±19.7	56.7±14.9** (↓26)
F1 generation males						
Body weight	23	59.9±10.0	64.7±9.0	61.6±6.5	58.5±9.7	44.5±7.3** (↓26)
	86	498.0±41.6	505.3±42.0	500.2±38.6	479.4±46.5	446.4±53.0** (↓10)
Body wt gain	23-86	438.1±37.0	440.6±37.3	438.6±38.5	425.2±40.6	401.6±49.4** (↓8)
F1 generation females						
Body weight	23	58.9±8.2	64.2±7.7* (↑9)	58.4±6.8	54.2±8.0* (↓8)	42.9±7.4** (↓27)
	79	271.2±32.9	280.1±22.5	278.8±25.1	261.8±26.2	247.4±30.6** (↓9)
	86	284.4±33.5	294.6±28.5	289.7±26.8	271.2±29.4	256.3±31.4** (↓10)
Body wt gain	23-86	225.4±31.8	230.4±26.1	231.2±25.0	217.2±27.1	213.8±28.7 (↓5)

a Data were obtained from Tables A3, A4, B5, B6, C3, C4, D5, and D6 on pages 87, 89, 208, 209, 403, 405, 545, and 546 of the study report; n=25-30. Percent difference from controls (calculated by reviewers) is presented parenthetically.

* Significantly different from controls at $p \leq 0.05$

** Significantly different from controls at $p \leq 0.01$

Pre-mating food consumption and food efficiency for the P and F1 generations are presented in Table 3b. In the P generation, overall (Days 1-70) absolute food consumption (g/rat/day) was decreased ($p \leq 0.01$) by 7-8% in both sexes at 2500 ppm. However, overall relative food consumption (g/kg/day) was similar to controls in both sexes at this dose. Overall food efficiency (%) was decreased by 11-19% in both sexes at this dose. In the F1 generation, overall (Days 23-86) absolute food consumption was decreased ($p \leq 0.01$) by 9% each in both sexes at 2500 ppm. Overall relative food consumption was increased ($p \leq 0.05$) by 6% in the males and was similar to controls in the females at this dose. Overall food efficiency was increased ($p \leq 0.05$) by 5% in the females and was similar to controls in the males at this dose. Additionally at 2500 ppm, sporadic changes ($p \leq 0.05$) were observed in weekly absolute and relative food consumption and food efficiency in both sexes and generations.

All other differences from controls noted in body weight, body weight gain, food consumption, or food efficiency in the P and F1 generations were considered unrelated to treatment because they were minor, sporadic, and/or unrelated to dose.

TABLE 3b. Mean (±SD) absolute food consumption (g/rat/day), relative food consumption (g/kg/day), and food efficiency (%) during pre-mating. [a]

Observation	Days	Dose (ppm)				
		0	75	300	1000	2500
P generation males						
Absolute FC	1-70	27.3±2.9	27.6±1.8	27.2±2.0	26.4±2.5	25.5±2.2** (↓7)
Relative FC	1-70	55.9±2.4	56.3±2.0	56.3±2.5	55.5±3.6	55.0±2.9
Efficiency	1-70	10.8±1.4	10.7±1.7	10.5±1.7	10.1±1.6	9.6±1.3** (↓11)
P generation females						
Absolute FC	1-70	19.4±2.0	20.1±2.9	20.2±2.3	18.8±2.1	17.8±1.6* (↓8)
Relative FC	1-70	71.4±8.3	72.0±8.5	72.4±7.5	70.3±5.9	69.3±4.8
Efficiency	1-70	5.7±1.4	6.0±1.2	6.2±1.6	5.5±1.3	4.6±1.1** (↓19)
F1 generation males						
Absolute FC	23-86	28.2±2.4	28.4±2.2	27.9±2.3	27.3±2.1	25.7±3.0** (↓9)
Relative FC	23-86	93.0±4.8	92.4±4.7	93.1±5.3	93.0±4.3	98.6±4.7** (↑6)
Efficiency	23-86	24.8±1.1	24.6±1.2	24.8±1.4	24.7±1.4	24.8±1.3
F1 generation females						
Absolute FC	23-86	20.6±2.0	21.6±2.3	21.3±1.6	19.9±1.8	18.8±1.7** (↓9)
Relative FC	23-86	105.5±6.9	105.9±8.8	105.5±6.0	106.3±5.4	109.2±6.1
Efficiency	23-86	17.1±1.0	16.9±1.2	17.2±1.2	17.2±1.2	17.9±1.6* (↑5)

a Data were obtained from Tables A5-A7, B11-B13, C5-C7, and D11-D13 on pages 91, 93, 95, 214-216, 407, 409, 411, and 551-553 of the study report; n=24-30. Percent difference from controls (calculated by reviewers) is presented parenthetically.

* Significantly different from controls at p≤0.05

** Significantly different from controls at p≤0.01

b. <u>Gestation</u>: Body weights, body weight gains, food consumption, and food efficiency data for the P and F1 generation females during gestation are presented in Table 4 below. In the P generation, at 2500 ppm, body weights were decreased (p≤0.05) by 7-8% throughout gestation and overall (GD 0-21) body weight gain was decreased by 8%, although it did not attain statistical significance. Additionally at this dose, absolute food consumption was decreased (p≤0.01) by 13% each during GD 0-7 and 7-14, and by 10% for the overall gestation period.

In the F1 generation, at 2500 ppm, body weights were decreased (p≤0.05) by 8% throughout gestation and overall body weight gain was decreased by 9%, although it did not attain statistical significance. Additionally at this dose, absolute food consumption was decreased (p≤0.05) by 8-10% during GD 0-7, 7-10, 10-14, and 18-21, and by 8% for the overall gestation period.

Relative food consumption and food efficiency were unaffected by treatment in the P and F1 generation during gestation at all doses. All other differences from controls noted in body weight, body weight gain, food consumption, or food efficiency in the P and F1 generations were considered unrelated to treatment because they were minor, sporadic, and/or unrelated to dose.

TABLE 4. Mean (±SD) body weights, body weight gains (g), absolute (g/rat/day), and relative (g/kg/day) food consumption during gestation. [a]

Observation	GD	Dose (ppm)				
		0	75	300	1000	2500
P generation females						
Body weight	0	303.5±24.8	318.2±26.0*(↑5)	314.8±29.8	299.6±22.5	283.2±16.2** (↓7)
	21	461.1±34.1	475.3±39.1	469.7±34.1	445.6±26.9	426.3±26.2** (↓8)
Body wt gain	0-21	157.6±24.3	157.1±26.0	154.1±19.9	145.1±19.8	145.5±20.0 (↓8)
Absolute FC	0-7	25.1±2.7	25.8±3.6	24.2±1.9	23.5±2.6* (↓6)	21.9±2.0** (↓13)
	7-14	25.7±3.4	26.2±2.7	25.4±1.8	24.8±2.5	22.3±2.3** (↓13)
	0-21	25.0±2.8	25.9±2.9	24.7±1.6	23.8±2.0	22.4±1.6** (↓10)
Relative FC	0-21	68.2±6.5	67.8±5.7	65.6±4.7	66.5±4.9	65.2±2.7
Efficiency	0-21	30.1±3.7	29.1±4.1	29.8±3.6	29.0±3.9	31.4±3.8
F1 generation females						
Body weight	0	287.7±31.4	300.6±32.2	299.6±29.5	278.1±27.3	264.5±32.7* (↓8)
	21	456.7±42.8	462.4±46.0	472.4±46.3	442.5±39.4	418.9±46.2** (↓8)
Body wt gain	0-21	169.4±19.4	163.5±20.1	172.9±29.8	163.8±23.4	153.9±21.0 (↓9)
Absolute FC	0-7	26.4±3.4	27.0±3.0	26.2±3.5	24.7±2.4	24.2±2.3* (↓8)
	18-21	24.9±3.3	25.3±3.0	25.5±4.1	24.4±3.0	22.4±3.6* (↓10)
	0-21	26.7±3.4	27.7±2.8	27.4±2.4	25.8±2.3	24.6±2.2* (↓8)
Relative FC	0-21	74.5±6.4	75.6±6.2	75.4±8.0	74.9±5.8	76.0±7.4
Efficiency	0-21	30.5±3.0	28.3±3.0	29.7±4.1	30.2±2.6	29.6±4.1

a Data were obtained from Tables B7, B8, B14-B16, D7, D8, and D14-D16 on pages 210, 211, 217-219, 547, 548, and 554-556 of the study report. Percent difference from controls (calculated by reviewers) is presented parenthetically.

* Significantly different from controls at p≤0.05

** Significantly different from controls at p≤0.01

c. <u>Lactation</u>: Body weights, body weight gains, food consumption, and food efficiency data for the P and F1 generation females during gestation are presented in Table 5 below. In the P generation, at 2500 ppm, body weights were decreased (p≤0.05) by 7-8% throughout lactation and body weight gains were increased (p≤0.05) by 177 and 223% during LD 15-22 and 1-22 (overall), respectively. Additionally at this dose, absolute food consumption was decreased (p≤0.05) by 16-20% during LD 5-8, 8-11, 11-15, 8-15, and 1-15, and relative food consumption was decreased (p≤0.05) by 10-12% during LD 8-15 and 1-15. Food efficiency was unaffected by treatment during lactation.

In the F1 generation, at 2500 ppm, body weights were decreased (p≤0.05) by 6-10% throughout lactation and body weight gains were increased by 48-168% during LD 5-8, 1-15, and 15-22 and by 304% for the overall (LD 1-22) lactation period. Additionally at this dose, absolute food consumption was decreased (p≤0.05) by 12% during LD 11-15, while relative food consumption was unaffected by treatment during lactation. Food efficiency was increased (p≤0.01) by 64% during LD 1-15.

At 1000 ppm, body weight gains were increased (p≤0.05) for the overall lactation period by 88% in the P females and by 127% in the F1 females. These increases were due to unusually large body weight gains in both generations on LD 5-8 (↑517% in the P females; ↑121% in the F1 females, and were not considered adverse.

All other differences from controls noted in body weight, body weight gain, food consumption, or food efficiency in the P and F1 generations were considered unrelated to treatment because they were minor, sporadic, and/or unrelated to dose.

TABLE 5. Mean (±SD) body weights, body weight gains (g), absolute (g/rat/day), and relative (g/kg/day) food consumption during lactation. [a]

Observation	LD	Dose (ppm)				
		0	75	300	1000	2500
P generation females						
Body weight	1	339.4±28.7	350.5±26.1	345.5±29.9	330.3±22.1	313.0±22.4**(↓8)
	15	369.4±16.9	377.4±25.9	368.9±24.9	365.6±22.7	344.4±25.6**(↓7)
	22	353.1±20.3	355.4±22.5	352.9±17.1	356.1±22.3	357.6±24.5
Body wt gain	1-22	13.7±21.5	4.9±16.6	7.4±20.0	25.8±22.0*(↑88)	44.2±15.5**(↑223)
Absolute FC	1-5	35.4±6.7	34.9±9.0	33.0±10.6	28.9±5.8** (↓18)	31.8±5.9
	5-8	39.0±8.6	42.1±6.5	39.3±8.6	41.0±10.0	32.6±6.6* (↓16)
	8-11	48.6±10.0	52.7±9.4	49.6±6.9	46.5±7.9	38.9±8.0** (↓20)
Relative FC	1-5	103.9±22.5	99.0±22.5	96.3±36.5	87.0±17.3**(↓16)	100.1±16.8
	8-15	153.7±26.6	159.9±17.6	158.2±18.7	150.2±18.9	134.6±21.6**(↓12)
	1-15 [b]	131.6±24.3	134.6±15.8	132.2±24.8	127.3±17.0	118.9±13.7* (↓10)
Efficiency	1-15	4.5±3.3	3.8±2.5	3.4±2.5	5.7±2.3	5.7±2.8
F1 generation females						
Body weight	1	337.8±39.8	350.1±33.4	347.2±32.2	326.6±27.5	304.5±32.2** (↓10)
	15	363.7±29.3	376.0±27.8	373.2±24.9	356.8±28.8	342.8±36.0* (↓6)
	22	348.1±23.3	363.4±23.7	364.4±28.8*(↑5)	349.8±24.9	345.7±33.6
Body wt gain	1-5	6.5±9.4	3.1±12.7	7.5±10.2	4.3±11.9	10.9±14.1
	5-8	4.7±8.0	6.4±8.4	6.4±8.8	10.4±7.4* (↑121)	12.6±12.0** (↑168)
	1-15	25.9±18.4	25.8±18.8	26.0±14.0	30.2±15.8	38.3±15.6* (↑48)
	15-22	-15.7±14.1	-12.6±14.6	-8.8±12.1	-7.0±14.8	2.8±17.0** (↑118)
	1-22	10.2±22.2	13.2±18.1	17.3±16.3	23.2±12.0*(↑127)	41.2±15.1** (↑304)
Absolute FC	1-5	38.0±8.4	37.4±5.5	41.4±7.5	35.2±5.5	38.4±8.7
	11-15	57.2±7.6	57.1±9.5	56.7±8.9	55.7±9.4	50.5±8.5* (↓12)
	1-15[b]	48.3±5.4	48.4±5.7	50.6±4.1	47.2±5.0	45.4±6.1
Relative FC	1-15 [b]	139.8±21.1	135.1±18.0	141.7±16.2	139.5±13.8	141.6±21.3
Efficiency	1-15	3.7±2.6	3.8±2.5	3.7±2.0	4.6±2.5	6.1±2.7** (↑64)

a Data were obtained from Tables B9, B10, B17-B19, D9, D10, and D17-D19 on pages 212, 213, 220-222, 549, 550, and 557-559 of the study report. Percent difference from controls (calculated by reviewers) is presented parenthetically.

b Because it was presumed that the pups began to consume maternal feed after LD 15, maternal food consumption values were not tabulated from LD 15-22.

* Significantly different from controls at $p \leq 0.05$

** Significantly different from controls at $p \leq 0.01$

3. <u>Test substance intake</u>: The mean test substance intake for both generations during pre-mating is considered to be representative of the achieved intake for the entire study (Table 6).

TABLE 6. Mean test substance intake (mg/kg/day in males/females) during pre-mating [a]					
Generation	Dose (ppm)				
	0	75	300	1000	2500
P generation	0/0	4.2/5.4	16.9/21.7	55.5/70.3	137.5/173.4
F1 generation	0/0	6.9/7.9	27.9/31.6	93.0/106.3	246.5/273.1

a Data were obtained from Tables A1, B1, C1, and D1 on pages 83, 201, 399, and 537 of the study report.

4. Reproductive function

a. Estrous cycle length and periodicity: There were no effects of treatment on estrous cycle duration or periodicity in the P or F1 generation females.

b. Sperm measures: There were no effects of treatment on sperm motility, counts, or morphology in either generation.

5. Reproductive performance: No treatment-related effects on the mating, fertility, or gestation indices were observed in either generation (Table 7). The significant differences ($p \leq 0.05$) in fertility index noted in the P generation females at 75, 300, and 1000 ppm were considered to be unrelated to treatment because they were greater than controls at 300 and 1000 ppm, were within the historical control ranges provided, and/or unrelated to dose. There were no effects of treatment on the pre-coital interval or gestation duration in either generation.

TABLE 7. Reproductive performance [a]

Parameter		Dose (ppm)				
		0	75	300	1000	2500
P generation						
Pre-coital interval (mated with female)						
(N/%)	Days 1-7	28/96.6	26/96.3	28/96.6	28/96.6	29/96.7
	Days 8-14	1/3.4	1/3.7	1/3.4	1/3.4	1/3.3
Pre-coital interval (mated by first male)						
(N/%)	Days 1-7	28/93.3	26/89.6	28/93.3	28/93.3	29/96.7
	Days 8-14	1/3.3	1/3.4	1/3.3	1/3.3	1/3.3
(mated by second male)	Days 15-21	1/3.3	2/6.9	1/3.3	1/3.3	0/0
Number paired	males	30	30	30	30	30
	females	30	30	30	30	30
Number mating	males	29	28	29	29	30
	females	30	30	30	30	30
Mating index (%)	males	96.7	93.3	96.7	96.7	100
	females	100	100	100	100	100
Fertility index (%)	males	86.2	82.1	100	96.6	90.0
	females	86.7	76.7*	100*	96.7*	90.0
Gestation index (%)		100	100	96.7	96.6	100
Gestation duration (mean # days)		22.5±0.6	22.6±0.6	22.7±0.5	22.7±0.5	22.7±0.5
F1 generation						
Pre-coital interval (mated with female)						
(N/%)	Days 1-7	27/96.4	29/96.7	26/92.8	25/92.6	27/100
	Days 8-14	1/3.6	1/3.3	2/7.1	2/7.4	0/0
Pre-coital interval (mated by first male)						
(N/%)	Days 1-7	27/93.1	29/96.7	26/86.7	25/86.2	27/96.4
	Days 8-14	1/3.4	1/3.3	2/6.7	2/6.9	0/0
(mated by second male)	Days 15-21	1/3.4	0/0	2/6.7	2/6.9	1/3.6
Number paired	males	30	30	30	30	29
	females	30	30	30	30	29
Number mating	males	28	30	28	28	28
	females	29	30	30	30	29
Mating index (%)	males	93.3	100	93.3	93.3	96.6
	females	96.7	100	100	100	100
Fertility index (%)	males	92.8	93.3	78.6	96.4	92.8
	females	93.1	93.3	80.0	93.3	93.1
Gestation index (%)		100	100	100	100	100
Gestation duration (mean # days)		22.8±0.8	22.8±0.6	22.8±0.6	22.8±0.6	23.2±0.6

a Data were obtained from Tables A8, B20, B21, C9, D21, and D22 on pages 97, 224, 225, 414, 562, and 563 of the study report.

* Significantly different from controls at $p \leq 0.05$

6. Parental postmortem results

a. Organ weights: Findings in the P generation males were limited to increased ($p \leq 0.05$) relative (to body weight) liver weights at 1000 and 2500 ppm (↑6 and 10%, respectively, Table 8a); however, the minor increase in the 1000 ppm males was not considered adverse. At 2500 ppm in the P generation females, the following differences ($p \leq 0.05$) in organ weights compared to controls were noted: (i) absolute and relative (to body and to brain weight) liver weights were increased by 6-10%; (ii) absolute and relative (to body and to brain weight) thymus weights were decreased by 29-32%; (iii) absolute and relative (to body and to brain weight) non-gravid uterus weights were decreased by 30-32%; (iv) absolute and relative (to body and to brain weight) pituitary weights were decreased by 13-20%; (v) absolute brain weight was decreased by 3%; and (vi) absolute and relative (to body weight) right ovary weights were decreased by 13% each. All other statistically significant differences noted in the P generation were considered unrelated to treatment because they were minor and/or were unrelated to dose.

TABLE 8a. Selected mean (±SD) absolute (g), relative (to body and to brain, %) organ weights in the P generation. [a]

Parameter		Dose (ppm)				
		0	75	300	1000	2500
P generation males						
Terminal BW (g)		633.3±69.3	641.2±55.5	634.1±55.2	619.7±56.8	603.2±60.0
Liver	Absolute	22.51±3.94	22.37±3.07	23.07±2.41	23.31±3.54	23.54±3.24
	Rel. (to body)	3.54±0.33	3.48±0.28	3.64±0.26	3.75±0.32*(↑6)	3.90±0.33** (↑10)
	Rel. (to brain)	970.0±174.3	974.0±134.4	1009.5±109.3	1035.2±161.9	1039.5±138.4
P generation females						
Terminal BW (g)		353.1±20.3	355.4±22.5	352.9±17.1	356.1±22.3	355.0±27.8
Liver	Absolute	17.35±1.59	16.87±1.79	16.75±1.56	16.95±1.62	18.42±1.80*(↑6)
	Rel. (to body)	4.916±0.398	4.746±0.412	4.750±0.422	4.756±0.260	5.191±0.325*(↑6)
	Rel. (to brain)	821.5±82.4	787.1±91.8	796.7±83.2	810.5±79.5	900.2±81.3**(↑10)
Thymus	Absolute	0.19±0.05	0.20±0.05	0.18±0.04	0.18±0.04	0.13±0.05**(↓32)
	Rel. (to body)	0.05±0.02	0.06±0.02	0.05±0.01	0.05±0.01	0.04±0.01**(↓30)
	Rel. (to brain)	9.1±2.4	9.3±2.4	8.7±1.9	8.6±2.0	6.5±2.7**(↓29)
Pituitary	Absolute	0.017±0.003	0.018±0.005	0.016±0.004	0.016±0.003	0.014±0.003**(↓18)
	Rel. (to body)	4.85±0.96	5.19±1.35	4.61±1.12	4.62±1.00	3.90±0.91**(↓20)
	Rel. (to brain)	0.8±0.2	0.8±0.2	0.8±0.2	0.8±0.2	0.7±0.2*(↓13)
Uterus	Absolute	0.65±0.16	0.57±0.11	0.61±0.16	0.57±0.13	0.44±0.14**(↓32)
	Rel. (to body)	0.183±0.05	0.161±0.03	0.174±0.05	0.161±0.04	0.124±0.04**(↓32)
	Rel. (to brain)	30.8±8.1	26.6±4.9	29.4±8.6	27.3±6.3	21.6±7.1**(↓30)
Brain	Absolute	2.12±0.09	2.15±0.08	2.11±0.10	2.09±0.06	2.05±0.08**(↓3)
	Rel. (to body)	0.600±0.04	0.604±0.04	0.599±0.04	0.589±0.04	0.579±0.05
Ovary, Right	Absolute	0.062±0.013	0.066±0.015	0.064±0.011	0.056±0.011	0.054±0.012*(↓13)
	Rel. (to body)	17.58±3.82	18.73±4.53	18.04±3.03	15.92±3.70	15.23±3.25*(↓13)
	Rel. (to brain)	2.9±0.6	3.1±0.7	3.0±0.6	2.7±0.5	2.6±0.6

a Data were obtained from Tables A10-A12 and B25-B27 on pages 99-104 and 231-233 of the study report. Percent difference from controls (calculated by reviewers) is presented parenthetically.
* Significantly different from controls at $p \leq 0.05$
** Significantly different from controls at $p \leq 0.01$

At 2500 ppm in the F1 generation males, the following differences ($p \leq 0.05$) in organ weights compared to controls were noted (Table 8b): (i) absolute brain weight was decreased by 5%; (ii) absolute spleen weight was decreased by 7%; (iii) absolute left and right testes weights were decreased by 5-7%; (iv) absolute thyroid/parathyroid weights were decreased by 9%;

and (v) relative (to body weight) liver weights were increased by 11%. The increases ($p \leq 0.05$) noted in relative (to body weight) left epididymis, left cauda epididymis, and seminal vesicles with fluid weights were not considered to be related to treatment because the absolute weights for these organs were generally comparable to controls.

In the F1 generation females at 2500 ppm, the following differences ($p \leq 0.05$ or NS) in organ weights compared to controls were noted: (i) absolute and relative (to body and/or brain weight) left and right ovary weights were decreased by 10-16%; (ii) absolute and relative (to body weight) thyroid/parathyroid weights were decreased by 11-12%; and (iii) relative (to body and brain weight) liver weights were increased by 6-8%. The increased ($p \leq 0.05$) relative (to body weight) non-gravid uterus weight noted at 2500 ppm was not considered to be related to treatment because the absolute weight for this organ was generally comparable to controls. All other statistically significant differences noted in the F1 generation were considered unrelated to treatment because they were minor, occurred unilaterally, and/or were unrelated to dose.

TABLE 8b. Selected mean (±SD) absolute (g), relative (to body and to brain, %) organ weights in the F1 generation parents. [a]

Parameter		Dose (ppm)				
		0	75	300	1000	2500
F1 generation males						
Terminal BW (g)		655.6±71.8	671.8±65.6	658.7±69.4	630.8±68.9	600.2±84.6**(↓8)
Liver	Absolute	26.67±4.03	27.06±3.58	26.48±3.48	26.72±4.57	27.26±5.33
	Rel. (to body)	4057±0.329	4.025±0.344	4.059±0.320	4.217±0.376	4.522±0.383**(↑11)
Brain	Absolute	2.31±0.12	2.30±0.10	2.30±0.08	2.27±0.13	2.20±0.10**(↓5)
	Rel. (to body)	0.356±0.041	0.344±0.034	0.352±0.037	0.364±0.041	0.372±0.047
Spleen	Absolute	0.98±0.13	1.00±0.12	0.96±0.12	0.92±0.12	0.91±0.15*(↓7)
	Rel. (to body)	0.149±0.016	0.149±0.016	0.146±0.020	0.147±0.023	0.151±0.025
Testis, Left	Absolute	1.96±0.16	1.92±0.17	1.92±0.17	1.88±0.18	1.82±0.11**(↓7)
	Rel. (to body)	0.302±0.038	0.287±0.038	0.293±0.035	0.301±0.040	0.309±0.043
Testis, Right	Absolute	1.93±0.16	1.92±0.19	1.91±0.17	1.84±0.18	1.82±0.10*(↓5)
	Rel. (to body)	0.297±0.041	0.289±0.042	0.291±0.032	0.294±0.035	0.309±0.044
Thyroid	Absolute	0.044±0.007	0.045±0.005	0.049±0.006*(↑11)	0.045±0.006	0.040±0.007*(↓9)
	Rel. (to body)	6.83±1.28	6.72±0.94	7.49±1.02*(↑10)	7.18±1.10	6.78±1.14
F1 generation females						
Terminal BW (g)		348.1±23.3	363.4±23.7	364.4±28.8*(↑5)	349.8±24.9	345.7±33.6
Liver	Absolute	17.55±2.02	17.24±2.31	18.10±2.12	16.80±1.78	18.53±2.22
	Rel. (to body)	5.04±0.46	4.74±0.56*(↓6)	4.96±0.43	4.81±0.44	5.36±0.33*(↑6)
	Rel. (to brain)	850.9±104.8	821.0±117.3	865.8±109.8	821.4±116.5	921.8±104.6*(↑8)
Thyroid	Absolute	0.035±0.007	0.037±0.006	0.037±0.007	0.032±0.006	0.031±0.006*(↓11)
	Rel. (to body)	10.15±1.78	10.27±1.69	10.23±1.84	9.03±1.72*(↓11)	8.91±1.55*(↓12)
Ovary, Left	Absolute	0.067±0.012	0.068±0.012	0.063±0.013	0.060±0.012*(↓10)	0.056±0.012**(↓16)
	Rel. (to body)	19.31±3.0	18.58±3.28	17.22±3.14*(↓11)	17.11±3.46*(↓11)	16.21±3.62**(↓16)
	Rel. (to brain)	3.3±0.6	3.2±0.6	3.0±0.6	2.9±0.7	2.8±0.6**(↓15)
Ovary, Right	Absolute	0.067±0.100	0.069±0.010	0.062±0.011	0.064±0.011	0.060±0.012*(↓10)
	Rel. (to body)	19.30±2.74	19.16±3.23	17.08±3.13*(↓12)	18.42±3.20	17.42±3.93 (↓10)
	Rel. (to brain)	3.2±0.5	3.3±0.5	3.0±0.5	3.1±0.6	3.0±0.6

a Data were obtained from Tables C11, C12, and D26-28 on pages 417-420 and 569-571 of the study report. Percent difference from controls (calculated by reviewers) is presented parenthetically.

* Significantly different from controls at $p \leq 0.05$

** Significantly different from controls at $p \leq 0.01$

b. Pathology

1) **Macroscopic examination:** There were no treatment-related gross findings in the P or F1 males or females.

2) **Microscopic examination:** In the P generation, microscopic findings were limited to minimal to moderate lymphoid atrophy of the thymus in the 2500 ppm females (11/30 treated vs. 0/30 controls, Table 9). No treatment-related microscopic lesions were observed in the P generation males at any dose or in the P generation females at up to 1000 ppm. No treatment-related microscopic lesions were observed in the F1 generation at any dose in either sex.

TABLE 9. Incidence of lymphoid atrophy of the thymus in P generation females. [a]

Severity	Dose (ppm)				
	0	75	300	1000	2500
Total	0	1	0	0	11
Minimal	0	1	0	0	4
Mild	0	0	0	0	4
Moderate	0	0	0	0	3

a Data were obtained from Table 1 on page 1291 of the study report, n=29-30.

B. OFFSPRING

1. **Viability and clinical signs:** Litter survival indices for the F1 and F2 litters are included in Table 10. No adverse clinical signs were observed in the F1 or F2 generation pups from birth to PND 22 at any dose. Pup sex ratio on PND 1 was unaffected by treatment in both generations. There were no treatment-related effects on the live birth, viability, or lactation indices at any dose in either generation. The decreased lactation index (98.6%; $p \leq 0.01$) noted at 2500 ppm in the F1 generation was minimal and within the historical control range provided (85.5-100% for 27 studies). The decreases ($p \leq 0.01$) noted in viability index at 75, 300, and 1000 ppm in the F2 generation were minimal and unrelated to dose.

TABLE 10. Litter parameters [a]					
Parameter	**Dose Group (ppm)**				
	0	**75**	**300**	**100**	**2500**
F1 litter					
Mean (±SD) implantation sites	15.8±3.0	16.0±2.2	16.6±3.0	15.6±2.1	15.6±1.7
Number born live	382	331	437	364	381
Number stillborn	4	0	1	6	2
Sex ratio on PND 1 (%♂)	49.2±16.4	52.7±12.5	53.0±13.5	54.4±12.6	49.7±11.6
Mean litter size, PND 1	14.7±2.9	14.4±2.8	15.1±2.8	13.0±2.4*	14.1±2.2
PND 5 (pre-cull)	14.5±2.9	14.1±2.8	14.7±2.7	12.8±2.4*	14.0±2.1
PND 5 (post-cull)	7.8±1.0	8.0±0.0	8.0±0.2	8.0±0.2	8.0±0.0
PND 8	7.8±1.0	8.0±0.0	8.0±0.2	8.0±0.2	8.0±0.2
PND 15	7.8±1.0	8.0±0.0	8.0±0.2	8.0±0.2	7.9±0.3
PND 22	7.8±1.0	8.0±0.0	8.0±0.2	8.0±0.2	7.9±0.4
Live birth index (%)	99.0	100.0	99.5	98.4	99.5
Number of females with total litter death	0	0	0	0	0
Viability index (%)	98.7	98.2	97.7	98.9	99.0
Lactation index (%)	100.0	100.0	100.0	100.0	98.6**
F2 litter					
Mean (±SD) implantation sites	16.2±2.4	16.0±2.7	16.6±2.2	16.0±1.8	16.0±3.0
Number born live	412	407	367	405	393
Number stillborn	1	1	3	4	1
Sex ratio on PND 1 (%♂)	47.2±12.7	52.8±17.2	50.4±17.4	51.0±12.9	50.4±14.7
Mean litter size, PND 1	15.2±2.6	14.5±4.0	15.3±2.2	14.5±2.0	14.6±3.3
PND 5 (pre-cull)	15.2±2.6	14.1±3.7	15.1±2.1	14.2±2.1	14.4±3.3
PND 5 (post-cull)	8.0±0.2	7.8±0.9	8.0±0.0	8.0±0.2	7.8±1.2
PND 8	8.0±0.2	7.8±0.9	8.0±0.2	8.0±0.2	1.7±1.2
PND 15	8.0±0.2	7.8±0.9	8.0±0.2	8.0±0.2	1.7±1.2
PND 22	7.9±0.3	7.8±0.9	8.0±0.2	7.9±0.3	1.7±1.2
Live birth index (%)	99.8	99.8	98.9	98.8	99.5
Number of females with total litter death	0	0	0	0	0
Viability index (%)	100.0	97.3**	98.6**	98.5**	99.2
Lactation index (%)	99.5	100.0	99.5	100.0	99.5

a Data were obtained from Tables B21, B22, D22, and D23 on pages 225-228 and 563-565 of the study report.

2. <u>Body weight</u>: Pup body weight data are presented in Table 11. At 2500 ppm, overall (PND 1-22) pup body weight gains (calculated by reviewers) were decreased in both generations by 17-26%. Mean pup body weight/litter at this dose was decreased ($p \leq 0.01$) by 13-23% on PND 8, 15, and 22 in the F1 generation and by 12-14% on PND 15 and 22 in the F2 generation.

At 1000 ppm in the F1 generation, mean pup body weight/litter was decreased ($p \leq 0.05$) by 7% on PND 22; however, this minor, transient decrease was not considered adverse.

Table 11. Mean (±SD) body weights/litter and body weight gains (g) in F1 and F2 generation pups. [a]						
Parameter		Dose Group (ppm)				
		0	75	300	1000	2500
F1 litters (n=23-29)						
Body weight	**PND 1**	6.8±0.9	7.0±1.0	6.8±0.7	7.0±0.9	6.8±0.6
	PND 8	17.5±2.6	18.3±2.5	16.8±2.6	16.6±2.6	15.2±2.0**(↓13)
	PND 22	54.6±8.3	58.7±7.2*(↑8)	54.7±5.5	50.6±7.3*(↓7)	42.3±5.0**(↓23)
Body weight gain[b]	**PND 1-22**	47.8	51.7	47.9	43.6	35.5 (↓26)
F2 litters (n=24-28)						
Body weight	**PND 1**	6.4±0.6	6.6±0.7	6.8±0.8	6.8±0.6	6.8±0.5
	PND 15	34.4±2.8	35.1±3.4	35.5±3.2	34.0±3.9	30.4±3.7**(↓12)
	PND 22	53.0±5.5	53.4±5.0	55.0±5.4	51.8±5.8	45.7±6.0**(↓14)
Body weight gain[b]	**PND 1-22**	46.6	46.8	48.2	45.0	38.9 (↓17)

a Data were obtained from Tables B22 and D23 on pages 228 and 566 of the study report. Percent difference from controls (calculated by reviewers) is presented parenthetically.
b Calculated by reviewers using data within this table.
* Significantly different from controls at $p \leq 0.05$
** Significantly different from controls at $p \leq 0.01$

3. Sexual maturation: In the F1 offspring, preputial separation and vaginal patency were unaffected by treatment. The average day on which preputial separation occurred was delayed ($p \leq 0.01$) and the mean body weight at criterion was decreased ($p \leq 0.01$) at 2500 ppm. However, both of these values were within the historical control ranges provided. Furthermore, when the individual body weight was used as a covariate in an analysis of covariance, statistical significance was no longer apparent. The average day on which vaginal patency occurred was also delayed ($p \leq 0.01$) at 2500 ppm. Despite the fact that the average day on which vaginal patency occurred was outside the historical control range (30.1-35.3 days), the delay was not statistically significant when individual body weight was used as a covariate in an analysis of covariance.

4. Offspring postmortem results

a) Organ weights: At 2500 ppm, the following decreases ($p \leq 0.05$) in both absolute and relative (to brain weight) organ weights were observed in the F2 generation (Table 12): (i) thymus (↓26-28% in both sexes); (ii) spleen (↓21-26%) in both sexes; and (iii) thyroid (↓33-34%) in the males and (↓12-20%) in the females. Both absolute and relative (to brain weight) thyroid weights were decreased by 17-25% in the 75, 300, and 1000 ppm F2 males; however, there was no clear dose response.

TABLE 12. Selected mean (±SD) absolute (g), relative (to brain weight, %) organ weights in the F2 generation. [a]						
Parameter		Dose (ppm)				
		0	75	300	1000	2500
F2 generation males						
Thymus	Absolute	0.25±0.05	0.23±0.04	0.24±0.05	0.24±0.04	0.18±0.05**(↓28)
	Rel. (to brain)	15.36±3.14	14.46±2.46	15.22±2.99	14.89±2.62	11.34±2.88**(↓26)
Spleen	Absolute	0.28±0.05	0.26±0.06	0.28±0.06	0.25±0.07	0.21±0.05**(↓25)
	Rel. (to brain)	17.14±3.30	16.93±3.95	17.56±3.66	15.73±3.85	13.58±3.36**(↓21)
Thyroid	Absolute	0.012±0.003	0.009±0.002**(↓25)	0.010±0.002**(↓17)	0.009±0.002**(↓25)	0.008±0.001**(↓33)
	Rel. (to brain)	0.733±0.158	0.596±0.119**(↓19)	0.594±0.103**(↓19)	0.549±0.097**(↓25)	0.483±0.087**(↓34)
F2 generation females						
Thymus	Absolute	0.25±0.06	0.22±0.05	0.24±0.06	0.23±0.04	0.18±0.04**(↓28)
	Rel. (to brain)	16.25±3.34	14.43±3.11	15.70±3.43	15.21±2.71	11.85±2.77**(↓27)
Spleen	Absolute	0.27±0.07	0.26±0.05	0.28±0.07	0.24±0.06	0.20±0.04**(↓26)
	Rel. (to brain)	17.18±4.02	17.08±2.89	18.22±4.13	15.50±3.25	13.32±2.65**(↓22)
Thyroid	Absolute	0.010±0.001	0.009±0.002	0.010±0.002	0.009±0.002	0.008±0.002*(↓20)
	Rel. (to brain)	0.633±0.073	0.590±0.119	0.660±0.157	0.601±0.121	0.554±0.159*(↓12)

a Data were obtained from Table D30 on pages 573-576 of the study report, n=24-28 litters. Percent difference from controls (calculated by reviewers) is presented parenthetically.
* Significantly different from controls at p≤0.05
** Significantly different from controls at p≤0.01

b) Pathology

1) Macroscopic examination: No macroscopic findings could be attributed to treatment in the F2 pups.

2) Microscopic examination: Thymic atrophy similar to what was observed in the P generation females at 2500 ppm was only observed in one F1 generation female at 2500 ppm and this single incidence was considered to be incidental. There were no treatment-related microscopic findings in the F2 pups.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: It was concluded that the parental and offspring LOAELs were 1000 ppm based on treatment-related effects on body weights at the end of lactation and immediately following weaning. The P generation females had increased body weight and body weight gains at the end of lactation and F1 generation offspring/adults had reduced body weights at the end of lactation and immediately following weaning. These weight effects were consistent with dose-related toxicity that was clearly evident at 2500 ppm. The reproductive toxicity NOAEL was 2500 ppm based on the absence of any treatment-related effects on mating, fertility, litter sizes, sex ratio, pup birth weights, and all other endpoints describing reproductive performance.

B. REVIEWER COMMENTS

1. PARENTAL ANIMALS:
The treatment-related effects produced by picoxystrobin were mainly seen in the 2500 ppm P and F1 parental rats. The effects were on body weights, body weight gains, food consumption, food efficiency changes, organ weight changes, and histopathology. The

body weights, body weight gains, food consumption, and food efficiency effects are tabulated below, and the details are shown in Tables 3a, 3b, 4 and 5.

Percent Change Relative to the Controls (%) in P & F1 Generation Rats at 2500 ppm								
Periods	**Body Weights**		**Body Weight Gains**		**Food Consumption**		**Food Efficiency**	
	Males	Females	Males	Females	Males	Females	Males	Females
	P Generation							
Pre-mating	↓4-7%	↓5-7%	↓18%	↓26%	↓7%	↓8%	↓11%	↓19
Gestation		↓7-8%		↓8%		↓10%		–
Lactation		↓7-8%		↑223%		↓16-20%		–
	F1 Parental Rats							
Pre-mating	10-26%↓	9-27%↓	↓8%	↓5%	↓9%	↓9%	–	↑5%
Gestation		↓8%		↓9%		↓8%		–
Lactation		↓6-10%		↑304%		↓12% at LD 11-15		↑64%

Additionally at 2500 ppm, the following differences in organ weights compared to controls were noted in the P generation females: (i) absolute and relative (to body and to brain weight) liver weights were increased by 6-10%; (ii) absolute and relative (to body and to brain weight) thymus weights were decreased by 29-32%; (iii) absolute and relative (to body and to brain weight) non-gravid uterus weights were decreased by 30-32%; (iv) absolute and relative (to body and to brain weight) pituitary weights were decreased by 13-20%; (v) absolute brain weight was decreased by 3%; and (vi) absolute and relative (to body weight) right ovary weights were decreased by 13% each. Findings in the P generation males were limited to increased relative (to body weight) liver weight (incr. 10%) at this dose. Microscopic findings were limited to minimal to moderate lymphoid atrophy in the thymus in the 2500 ppm females (11/30 treated vs. 0/30 controls).

The LOAEL for parental toxicity is 2500 ppm (137.5/173.4 mg/kg/day in males/females, respectively) based on decreases in body weight, body weight gain, and food consumption in the P and F1 generation during pre-mating; increased body weight gains in the P and F1 females at the end of the lactation period; organ weight differences; and minimal to moderate lymphoid atrophy in the thymus in P generation females. The NOAEL is 1000 ppm (55.6/70.3 mg/kg/day in males/females, respectively).

2. **<u>OFFSPRING</u>:** There were no treatment-related effects on: mortality; clinical signs; live birth, viability, and lactation indices; or pup sex ratio for either generation. There were no treatment-related gross or microscopic findings in the F1 or F2 pups.

   At 2500 ppm, overall (PND 1-22) pup body weight gains were decreased in both the F1 and F2 generations by 17-26%. Mean pup body weights/litter at this dose were decreased by 13-23% on PND 8, 15, and 22 in the F1 generation and by 12-14% on PND 15 and 22 in the F2 generation. Organs weights such as thymus, spleen, and thyroid were decreased in F1 and F2 pups.

   The LOAEL for offspring toxicity is 2500 ppm (137.5/173.4 mg/kg/day in males/females, respectively) based on decreased mean pup body weights/litter and body weight gains in the F1 and F2 generations and decreased organ weights including spleen, thymus, and thyroid. The NOAEL is 1000 ppm (55.6/70.3 mg/kg/day in males/females, respectively).

3. **REPRODUCTIVE TOXICITY:** There were no effects of treatment in either generation on: estrous cycle; sperm parameters; mating, fertility, litter sizes, sex ratio, pup birth weights, or gestation indices; pre-coital interval; or gestation duration.

   The LOAEL for reproductive toxicity was not observed. The NOAEL is 2500 ppm (137.5/173.4 mg/kg/day in males/females, respectively) (HDT).

   This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.3800; OECD 416) for a two-generation reproduction study in the rat.

IV. STUDY DEFICIENCIES: No study deficiencies were noted.